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Evidence of the involvement of the monoaminergic systems in the antidepressant-like effect of *Aloysia gratissima*

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ABSTRACT

Ethnopharmacological relevance: Aloysia gratissima (Verbenaceae) is an aromatic plant distributed in South America and, employed in folk medicine for the treatment of nervous systems illness, including depression. The neuroprotective and antidepressant-like activities of the aqueous extract of *Aloysia gratissima* (AE) administered orally has already been demonstrated.In this study the involvement of monoaminergic systems in the antidepressant-like effect of the AE was investigated.

Materials and methods: The implication of the monoaminergic systems in the antidepressant-like activity of *Aloysia gratissima* was evaluated using different pharmacological antagonists that were administered previously to the acute oral administration of AE (10 mg/kg). The antidepressant-like effect was assessed in the TST and locomotor activity was evaluated in the open-field test in mice.

Results: The anti-immobility effect elicited by AE in the TST was prevented by the pre-treatment of mice with the antagonists, NAN-190 (5-HT_{1A} receptor), ketanserin (5-$HT_{2A/2C}$ receptor), prazosin (α_1-adrenoceptor), yohimbine (α_2-adrenoceptor), SCH23390 (dopamine D_1 receptor), or sulpiride (dopamine D_2 receptor).

Conclusions: These results indicate that the antidepressant-like effect of AE in the TST is dependent on its interaction with the serotonergic (5-HT_{1A} and 5-$HT_{2A/2C}$), noradrenergic (α_1 and α_2-adrenoceptors) and dopaminergic (D_1 and D_2 receptors) systems, suggesting that this specie might act as a new potential resource for developing antidepressants to treat depressive disorders.

1. Introduction

Depression is a common disorder affecting over 120 million people worldwide and recent epidemiological surveys conducted in general populations have found that the lifetime prevalence of depression is in the range of 10–15% (Lépine and Briley, 2011). In spite of the introduction of the tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs) and specific serotonin–noradrenaline reuptake inhibitors (SNRIs), depression continues to be a major medical problem. However, antidepressant treatments largely have weaknesses such as slow onset and severe side effects (Dhingra and Parle, 2012).

Because of these problems individuals are seeking alternatives to pharmaceutical medications and herbal therapies may be effective alternative therapeutic tools for the treatment of depression, as in the case of St. John's wort (Linde and Knüppel, 2005). The search for novel pharmacotherapy from medicinal plants for depression has increased markedly over the past decades (Zhang, 2004; Sarris et al., 2011). Importantly, it is worth mentioning that new potential treatments for depression seems to act through a mechanism which does not differ significantly with respect to that of "classical" antidepressants such as inhibition of monoamine reuptake (such as serotonin, dopamine and noradrenaline), augmentation of binding and sensitization of serotonin receptors, inhibition of monoamine oxidase, and modulation of neuroendocrine system (Machado et al., 2007; Sarris et al., 2011).

Recently, the usage of traditional herbs has supported new alternatives for the treatment of depression and there is a growing abundance of preclinical and clinical studies which reveal a range of complex psychotropic activity from herbal medicines potentially beneficial for treating certain psychiatric conditions, most notably depression (Sarris et al., 2011). Therefore, the search for new antidepressant drugs is urgent in exploring more promising antidepressant although, the lack of wide scientific research involving therapeutic potential, safety, and quality of herbal extracts (Calixto, 2005; Sarris et al., 2011) deserves immediate attention.

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Aloysia gratissima is an aromatic native plant to southern Brazil, belonging to the Verbenaceae family which is widely distributed in subtropical regions of South America mainly Brazil, Paraguay, and Argentina. *Aloysia gratissima* has been investigated for virucidal (García et al., 2003), nematicidal (Duschatzky et al., 2004), fungicidal (Dellacasa et al., 2003), antioxidant (Rosas-Romero and Saavedra, 2005; Zeni et al., 2013), antibacterial, and antiedematogenic (Vandresen et al., 2010) activities and recently, antidepressant-like and neuroprotective effects by our research group (Zeni et al., 2011).

The ethnopharmacological purposes for *Aloysia gratissima* such as headache, bronchitis, and nervous systems disorders including symptoms related to depression, e.g., changes of humor and sadness have been reported by several authors (Del Vitto et al., 1997; Souza and Wiest, 2007; Vendruscolo et al., 2005; Arias Toledo, 2009). Considering all information, this study was aimed at extending the study with *Aloysia gratissima* by investigating a possible participation of the monoaminergic systems in the antidepressant-like effect of an acute administration of AE in the tail suspension test (TST) in mice.

2. Material and methods

2.1. Plant material and production of aqueous extract

Stems and leaves of *Aloysia gratissima* (Gill. et Hook) Tronc. (Verbenaceae) were collected in Guabiruba, Santa Catarina, identified and authenticated taxonomically at Regional University of Blumenau. A voucher specimen (Excicata number 2658) was deposited in the Dr. Roberto Miguel Klein Herbarium for future reference in Regional University of Blumenau, Santa Catarina, Brazil. The procedure to obtain the aqueous extract from *Aloysia gratissima* (AE) and phytochemical profile were recently described (Zeni et al., 2013). In summary, dried powdered material (2.5 g) was extracted with boiling water (150 ml) for 5 min. The extract obtained was lyophilized and stored and, suspended in distilled water at the time of the administration (yield: 25.71%).

2.2. Animals

Swiss male mice (60–70 days old weighing 30–40 g) were maintained at 22–23 °C with free access to water and food, under a 12:12 h light: dark cycle (lights on at 7:00 h). All manipulations were carried out between 9:00 and 16:00 h, with each animal used only once. The experiments were performed after approval of the protocol by the Ethics Committee of the Federal University of Santa Catarina and all efforts were made to minimize animal suffering.

2.3. Drugs and treatment

The following drugs were used: Ketanserin tartarate, 1-(2-methoxyphenyl)-4[-(2-phthalimido)butyl]piperazine (NAN-190), sulpiride, prazosin, yohimbine, (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-enzazepinehydrochloride (SCH23390) (all from Sigma Chemical Company, St. Louis, MO, U.S.A.). Drugs were dissolved in saline except NAN-190 that was diluted in saline with 1% Tween 80. Sulpiride and prazosin were diluted in saline with 5% dimethylsulfoxide (DMSO). Appropriate vehicle-treated groups were also assessed simultaneously. All drugs were administered by intraperitoneal (i.p.) route, except SCH23390 that was administered by subcutaneous (s.c.) route.

The AE (10 mg/kg) was dissolved in distilled water and administered acutely by oral route (p.o.) by gavage, 60 min before the TST or the open-field test. The dissolution of the extract was freshly done from the lyophilized powdered immediately before its administration. A control group received equal volume of distilled water.

To address some of the mechanisms by which the extract causes antidepressant-like action in the TST, animals were treated with different drugs. The experiments were performed to assess the interaction of the extract with the monoaminergic system. For that, mice were pre-treated with vehicle, NAN-190 (0.5 mg/kg, a 5-HT_{1A} receptor antagonist), ketanserin (5 mg/kg, a 5-$HT_{2A/2C}$ receptor antagonist), prazosin (1 mg/kg, an α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, an α_2-adrenoceptor antagonist), SCH23390 (0.05 mg/kg, a dopamine D_1 receptor antagonist), or sulpiride (50 mg/kg, a dopamine D_2 receptor antagonist), and 30 min later they received vehicle or extract (10 mg/kg, p.o.) before being tested in the TST after 60 min, as described previously (Binfaré et al., 2009; Machado et al., 2009).

2.4. Behavioral tests

2.4.1. Tail suspension test (TST)

The tail suspension test has become one of the most widely used models for assessing antidepressant-like activity in mice. The test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Mice were considered immobile only when they hung passively and completely motionless. The immobility time was recorded by an observer blind to the drug treatment (Rodrigues et al., 2002; Zeni et al., 2011).

2.4.2. Open-field test

The ambulatory behavior was assessed in an open-field test as described previously (Rodrigues et al., 2002; Rosa et al., 2008). The apparatus consisted of a wooden box measuring $40 \times 60 \times 50$ cm^3 high. The floor of the arena was divided into 12 equal rectangles. At the start of the each trial, a mouse was placed in the left corner of the field and was allowed to freely explore the arena. The number of rectangles crossed with all paws (crossing) was counted in a 6-min session. The light was maintained at minimum to avoid anxiety behavior. The apparatus were cleaned with a solution of 10% ethanol between tests in order to hide animal clues.

The doses of the drugs used were selected on the basis of literature and are previously reported not to increase locomotor activity (Redrobe and Bourin, 1997; Kaster et al., 2005; Machado et al., 2007; Binfaré et al., 2009).

2.5. Data analysis

Comparisons between treatment groups and control were performed by two-way analysis of variance (ANOVA) followed by Tukey's HSD test when appropriate. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. Involvement of the serotonergic system

The result presented in Fig. 1A shows that the pre-treatment of mice with NAN-190 (0.5 mg/kg, a 5-HT_{1A} receptor antagonist, i.p.) on the anti-immobility effect of AE (10 mg/kg, p.o.) in the TST. The pre-treatment of mice with NAN-190 was able to reverse the

antidepressant-like effect of AE. A two-way ANOVA revealed significant differences of AE treatment [$F(1,20)=6.35$, $P<0.05$] and NAN-190 × extract interaction [$F(1,22)=21.59$, $P<0.01$], but not NAN-190 pre-treatment [$F(1,22)=2.74$, $P=0.11$]. The administration of NAN-190 alone or in combination with AE did not affect ($P>0.05$) the ambulation in the open-field test (Fig. 1B). Fig. 1C shows the influence of pre-treatment of mice with ketanserin (5 mg/kg, a 5-$HT_{2A/2C}$ receptor antagonist, i.p.) on the anti-immobility effect of AE in the TST. A two-way ANOVA revealed significant differences of ketanserin pre-treatment [$F(1,22)=10.21$, $P<0.01$], AE treatment [$F(1,22)=4.80$, $P<0.05$], and ketanserin × AE interaction [$F(1,22)=14.05$, $P<0.01$]. Post hoc analyses indicated that the pre-treatment with ketanserin reversed the decrease in immobility time produced by the AE in the TST. The administration of ketanserin alone or in combination with AE did not affect ($P>0.05$) the ambulation in the open-field (Fig. 1D).

3.2. Involvement of the noradrenergic system

Fig. 2A shows that the pre-treatment of mice with prazosin (1 mg/kg, an α_1-adrenoceptor antagonist, i.p.) prevented the antidepressant-like effect of the AE (10 mg/kg, p.o.) in the TST. The two-way ANOVA revealed significant differences of prazosin pre-treatment [$F(1,20)=25.67$, $P<0.01$], extract treatment [$F(1,20)=10.15$, $P<0.01$], and prazosin pre-treatment × extract treatment interaction [$F(1,20)=25.88$, $P<0.01$]. The administration of prazosin alone or in combination with AE did not affect ($P>0.05$) the ambulation in the open-field (Fig. 2B). Fig. 2C shows the effect of pre-treatment of mice with yohimbine (1 mg/kg, an α_2-adrenoceptor antagonist, i.p.) on the reduction in the immobility time elicited by the administration of AE (10 mg/kg, p.o.). The pre-treatment of mice with yohimbine was able to reverse the antidepressant-like effect of AE. A two-way ANOVA revealed significant differences of yohimbine pre-treatment [$F(1,20)=10.75$, $P<0.01$], AE treatment [$F(1,20)=18.08$, $P<0.01$], and yohimbine pre-treatment × AE treatment interaction [$F(1,20)=27.30$, $P<0.01$]. The administration of yohimbine alone or in combination with AE did not affect ($P>0.05$) the ambulation in the open-field (Fig. 2D).

3.3. Involvement of the dopaminergic system

Fig. 3A shows that the anti-immobility effect of AE (10 mg/kg) was significantly prevented by pre-treatment of mice with SCH23390 (0.05 mg/kg, a dopamine D_1 receptor antagonist, s.c.). A two-way ANOVA revealed significant differences of SCH23390 pre-treatment [$F(1,22)=8.82$, $P<0.01$] and extract treatment [$F(1,22)=45.54$, $P<0.01$], and SCH23390 pre-treatment × extract treatment interaction [$F(1,22)=5.18$, $P<0.05$]. The administration of SCH23390 alone or in combination with AE did not affect ($P>0.05$) the ambulation in the open-field (Fig. 3B). The pre-treatment of the animals with sulpiride (50 mg/kg, a D_2 receptor antagonist, i.p.) was also able to prevent the anti-immobility effect of AE in the TST (Fig. 3C). The two-way ANOVA revealed significant differences of sulpiride pre-treatment [$F(1,24)=58.54$, $P<0.01$], extract treatment [$F(1,24)=22.77$, $P<0.01$], and sulpiride pre-treatment × extract treatment interaction [$F(1,24)=42.78$, $P<0.01$]. Post hoc analyses indicated that the pre-treatment of mice with SCH23390 and sulpiride prevented the decrease in immobility time in the TST produced by the administration of the extract. The administration of sulpiride alone or in combination with AE did not affect ($P>0.05$) the ambulation in the open-field (Fig. 3D).

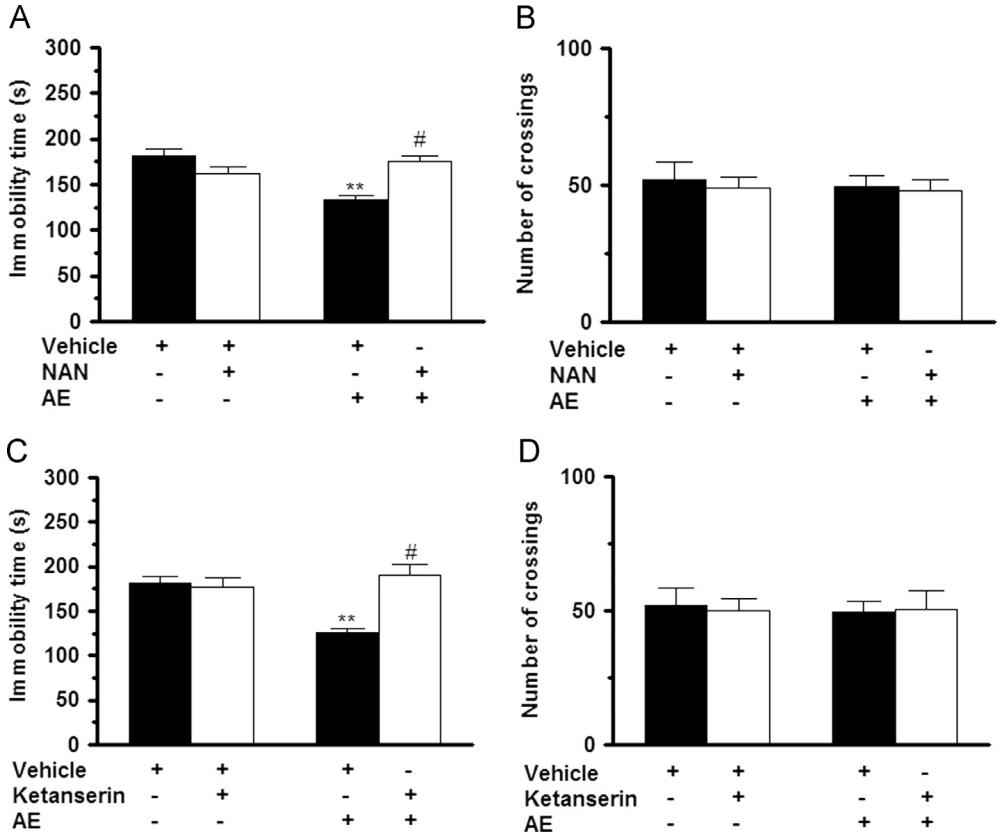

Fig. 1. Effect of the pre-treatment of mice with NAN-190 (0.5 mg/kg, i.p.) or ketanserin (5 mg/kg, i.p., panels A and C, respectively) on the anti-immobility effect of AE (10 mg/kg, p.o.) in the TST, and the number of crossings in the open-field test (panels B and D). Each column represents the mean ± S.E.M. of 6–7 animals. $^{**}P<0.01$ compared with the vehicle-treated control. $^{\#}P<0.01$ as compared with the extract alone.

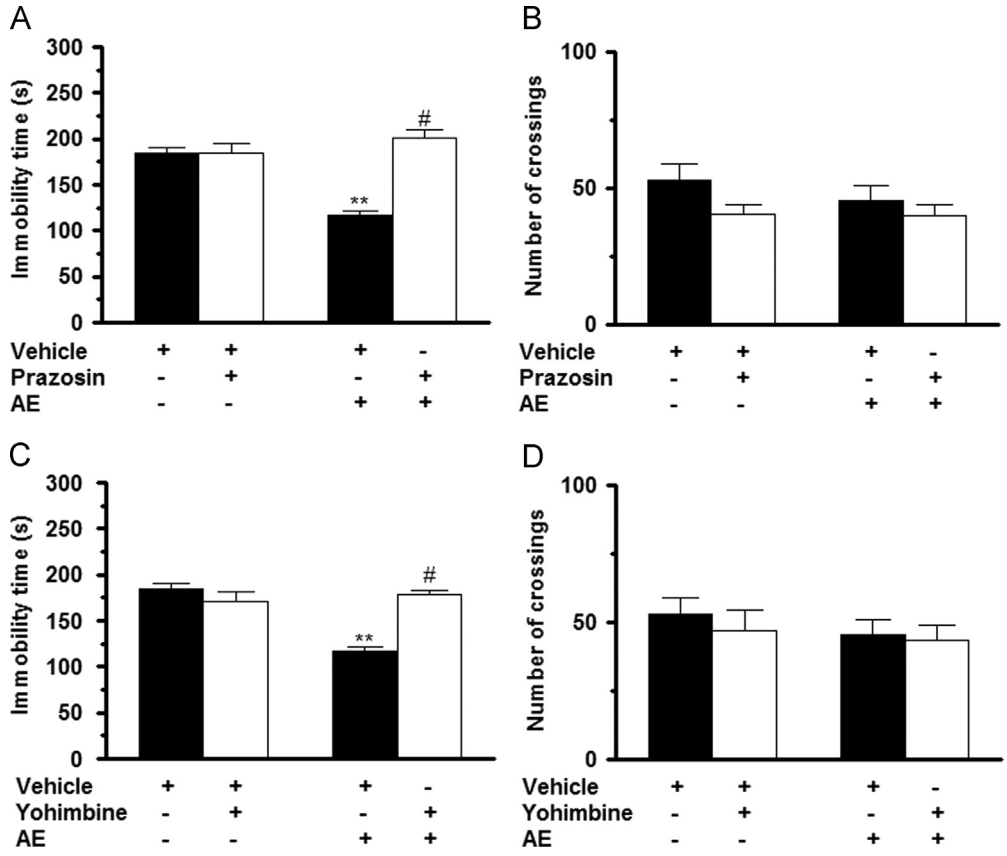

Fig. 2. Effect of the pre-treatment of mice with prazosin (1 mg/kg, i.p., panel A), yohimbine (1 mg/kg, i.p., panel C) on the anti-immobility effect of the aqueous AE (10 mg/kg, p.o.) in the TST and the number of crossings in the open-field test (panels B and D). Each column represents the mean ± S.E.M. ($n=6$). $^{**}P<0.01$ as compared with the vehicle-treated control. $^{\#}P<0.01$ as compared with the extract alone.

4. Discussion

The monoaminergic hypothesis of depression postulates that the major neurochemical process in depression is the impairment of monoaminergic neurotransmission and the concomitant decrease on extracellular concentration of noradrenaline and/or serotonin (Schildkraut, 1965). Most of the prescribed antidepressants inhibit serotonin or noradrenaline reuptake and/or monoamine oxidase inhibitors act by increasing the synaptic availability of these neurotransmitters (Montgomery, 1999; Taylor and Stein, 2006). The dopaminergic system is also an important target implicated in the regulation of mood disorders (Dailly et al., 2004), as preclinical and clinical studies have indicated a diminished dopamine turnover in depression (Elhwuegi, 2004; Millan, 2004).

This study intends to expand previous analysis on the possible mechanisms involved in the antidepressant-like effects of AE in the TST, since the TST is a well-characterized behavioral model predictive of antidepressant activity that is sensitive to antidepressants from different pharmacological classes (Steru et al., 1985; Cryan et al., 2005) and modulated by serotonergic, noradrenergic, and dopaminergic systems, among others (Cryan et al., 2005; O'Leary et al., 2007).

Aloysia gratissima has been reported to exhibit antidepressant-like effect in the TST, which is mediated through of the inhibition of the glutamatergic system (Zeni et al., 2011). Furthermore, the major polyphenolic compound of *Aloysia gratissima*, ferulic acid (Zeni et al., 2013), was also reported to produce an antidepressant-like effect (Zhang et al., 2011; Zeni et al., 2012) dependent on the serotonergic system. Of note, Poleszak et al. (2011) reported that the interaction with serotonergic system is necessary for the on antidepressant-like activity of glycine/NMDA site ligands.

In this study, the involvement of monoaminergic systems in the mechanism of AE antidepressant-like effect was assessed by treating mice with several pharmacological antagonists in order to investigate their influence on the anti-immobility action of AE in the TST. The results indicate that the effect of AE in the TST was reversed by pre-treatment of mice with the 5-HT_{1A} antagonist, NAN-190, suggesting the participation of 5-HT_{1A} receptors in the antidepressant-like effect of AE. Considering that Foong and Bornstein (2009) showed that NAN-190 also blocks α_2-adrenoceptors in the guinea pig, one possibility is that the ability of NAN-190 to abolish the antidepressant-like effect of AE may be due to, at least in part, the involvement of α_2-adrenoceptors. Pharmacologic and genetic studies have demonstrated that the serotonergic system plays a central role in the pathophysiology and etiology of depression (Ansorge et al., 2007) and a major role in the action of antidepressants (Millan, 2004). In accordance with Machado et al. (2009) and Binfaré et al. (2009) the pre-treatment with the NAN-190 also abolished the anti-immobility effect elicited by *Rosmarinus officinalis* L. or ascorbic acid, respectively.

The antidepressant-like effect of AE was also prevented by the pre-treatment with ketanserin, a 5-$HT_{2A/2C}$ receptor antagonist. Preclinical and clinical studies have reported a key role for 5-HT_2 receptors in the pathology of depression, as well as the action of many antidepressants (Cryan and Leonard, 2000; Boothman et al., 2006). A similar result was reported in previous studies from our research group in which ketanserin was able to reverse the antidepressant-like effect in the TST of extracts from *Schinus molle* L. (Machado et al., 2007), *Rosmarinus officinalis* L. (Machado et al., 2009), and *Tabebuia avellanedae* L. (Freitas et al., 2010). Thus, the present study indicates that the antidepressant-like effect of the

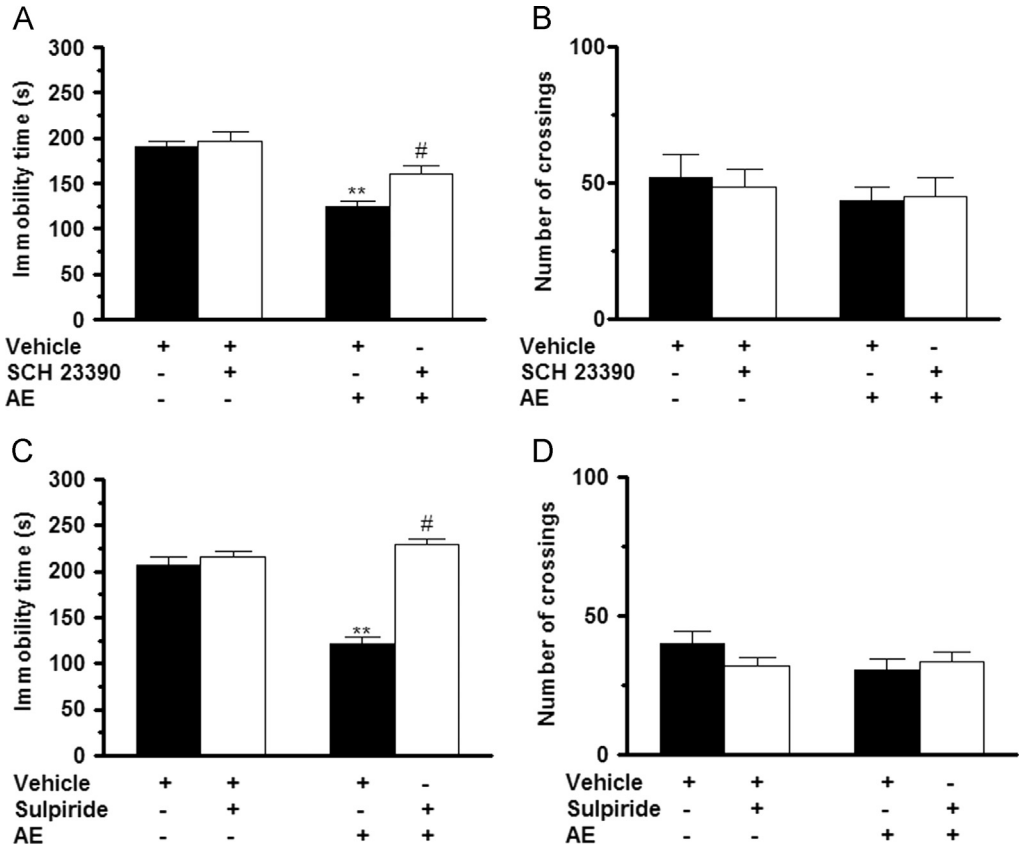

Fig. 3. Effect of the pre-treatment of mice with SCH23390 (0.05 mg/kg, s.c., panel A), and sulpiride (50 mg/kg, i.p., panel C) on the anti-immobility effect of the AE (10 mg/kg, p.o.) in the TST and the number of crossings in the open-field test (panels B and D). Each column represents the mean ± S.E.M. (n=6–7) $^{**}P < 0.01$ as compared with the vehicle-treated control. $^{\#}P < 0.01$ as compared with the extract alone.

AE seems to be mediated by the interaction with 5-HT_{2A} and/or 5-HT_{2C} receptors.

The role of noradrenaline on the pathophysiology of depression has been extensively investigated, since some antidepressant drugs increase the synaptic concentration of noradrenaline and they might act directly at noradrenergic receptors (Elhwuegi, 2004). Depression is associated with a hypofunction of the noradrenergic system and some antidepressants such as noradrenaline reuptake inhibitors or monoamine oxidase inhibitors act by increasing the synaptic availability of noradrenaline (Montgomery, 1999; Taylor and Stein, 2006).

In our study, the pre-treatment of mice with prazosin (an α_1-adrenoreceptor antagonist) and yohimbine (an α_2-adrenoreceptor antagonist) was able to block the antidepressant-like effect of AE. This result indicates that AE could exert its effect in the TST by interacting with both α_1 and α_2-adrenoceptors. It has been demonstrated that the pre-treatment of mice with prazosin was also able to reverse the antidepressant-like effect of the extract of *Rosmarinus officinalis* (Machado et al., 2009) in the TST. In addition, the antidepressant-like effects of *Schinus molle* (Machado et al., 2007) or *Tabebuia avellanedae* (Freitas et al., 2010), as well as the ascorbic acid in the TST (Binfaré et al., 2009) were reversed by the α_2-adrenoceptor antagonist yohimbine. Accordingly, there is compelling evidence for the role of α_1 and α_2-adrenoceptors in the mechanism of action of antidepressant agents as desipramine and amitriptyline (Kitada et al., 1986; Millan, 2004).

In the present work we have also observed that the selective dopamine D_1 receptor antagonist SCH23390 and the dopamine D_2 receptor antagonist sulpiride abolished the anti-immobility effects of AE in the TST. Indeed, our results point to a participation of both dopamine D_1 and D_2 receptors in the antidepressant-like effects of the extract investigated. This result is in agreement with the well-known relationship between dopamine neurotransmission and depression extensively reported in preclinical and clinical studies (Elhwuegi, 2004; Millan, 2004). Depressed and/or suicidal patients present reduced levels of dopamine and its metabolite homovanillic acid when compared to non-depressed ones (Hamner and Diamond, 1996; Papakostas, 2006). Furthermore, findings indicated that there is an increased dopamine D_2/D_3 receptor binding (Klimek et al., 2002) and reduced dopamine transporter activity (Meyer et al., 2001; Klimek et al., 2002) in depressive patients. Additionally, reports suggest that severity of depression is inversely correlated with central nervous system dopamine metabolite levels (Papakostas, 2006).

Some studies have indicated that the dopamine D_1 receptor antagonist SCH23390 prevented the antidepressant-like effect of some antidepressant agents in the FST and TST (Yamada et al., 2004; Hirano et al., 2007). Furthermore, preclinical data indicate that dopamine D_2 receptors are related to the anti-immobility action of antidepressants in the FST (Yamada et al., 2004). Thus, our results suggest that the effect of AE in the TST may depend on activation of both dopamine D_1 and D_2 receptors. It has been demonstrated that pre-treatment of mice with prazosin was able to reverse the antidepressant-like effect of the extract of *Rosmarinus officinalis* (Machado et al., 2009). In addition, similar to our results the antidepressant-like effects of *Schinus molle* (Machado et al., 2007) and *Tabebuia avellanedae* (Freitas et al., 2010) were reversed by SCH23390.

Drugs inhibiting the uptake of serotonin, noradrenaline, and dopamine (triple reuptake inhibitors) that have been recently developed could produce a more rapid onset of action and possess greater efficacy than traditional antidepressants (Chen and

Skolnick, 2007). Literature data have shown the potential of several herbal extracts and constituents as antidepressant agents, whose mechanism of action involves a simultaneous interaction with the monoaminergic systems (Rodrigues et al., 2002; Zhang, 2004; Machado et al., 2007, 2009; Binfaré et al., 2009; Freitas et al., 2010). In this study, AE exerts antidepressant-like effect through the involvement of serotonergic, noradrenergic and dopaminergic systems. Indeed, ferulic acid, the major polyphenolic compound of AE inhibits serotonin reuptake in hippocampus of rats and it has been proposed that the inhibition of noradrenaline and dopamine receptors is implicated in its anti-immobility effect (Zhang et al., 2011).

Similar to the findings with ascorbic acid reported by Binfaré et al., (2009) and Moretti et al. (2011), the present study has also demonstrated that the administration of AE produced an antidepressant-like effect in the TST. Therefore, both AE and ascorbic acid act through a mechanism that is dependent on their interaction with the monoaminergic systems, N-methyl-D-aspartate (NMDA) receptors and the L-arginine–nitric oxide (NO)–cyclic guanosine monophosphate (cGMP) pathway (Zeni et al., 2011). Furthermore, an additional study aiming at clarifying the mechanism of action by which AE induces antidepressant-like effect is essential, such as, measuring the reuptake of monoamines or activity on transporters.

The results herein shown reveal that AE is an effective antidepressant-like complex matrix with meaningful effects on the monoaminergic systems as well as on the L-arginine–NO pathway, corroborating the folk medicine usage of that plant species in southern Brazil.

5. Conclusion

In conclusion, the present study provides additional evidence indicating that the AE produces a specific antidepressant-like effect in the TST through the interaction with the serotonergic (5-HT_{1A} and 5-$HT_{2A/2C}$ receptors), noradrenergic (α_1 and α_2-adrenoceptors) and dopaminergic (D_1 and D_2 receptors) systems. Thus, our results suggest that AE shares with established antidepressants some mechanisms of action. At least at a preclinical level the findings reported here is a relevant contribution to validate the traditional use of *Aloysia gratissima*.

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